

WHAT IS CLAIMED IS:

1. A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:
  - 5 producing in a yeast cell a first fusion protein and a second fusion protein, said first fusion protein having said first test polypeptide and an N-intein, said second fusion protein having said second test polypeptide and a C-intein, wherein at least one of the two fusion proteins has an inactive reporter capable of being converted to an active reporter protein upon trans-splicing through said N-intein and said C-intein; and
  - 10 determining the production of said active reporter protein.
2. A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:
  - introducing into an yeast cell a first chimeric gene and a second chimeric gene,
  - 15 said first chimeric gene encoding a first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide fused to the N-terminus of an N-intein, said second chimeric gene encoding a second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive
  - 20 reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein;
  - expressing said first fusion protein and said second fusion protein in said yeast cell; and
  - 25 detecting said active reporter protein.
3. The method of Claim 2, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.
- 30 4. The method of Claim 2, wherein said yeast cell is a diploid cell and said step of introducing into said yeast cell said first chimeric gene and said second chimeric

gene comprises mating a first haploid yeast cell having said first chimeric gene with a second haploid yeast cell having said second chimeric gene.

5        5.        The method of Claim 2, wherein said first test polypeptide is fused to the C-terminus of said N-intein in said first fusion protein, and said second test polypeptide is fused to the N-terminus of said C-intein in said second fusion protein.

10       6.        The method of Claim 2, wherein said first test polypeptide is fused to the N-terminus of said first inactive reporter polypeptide in said first fusion protein, and said second test polypeptide is fused to the N-terminus of said C-intein in said second fusion protein.

15       7.        The method of Claim 2, wherein said first test polypeptide is fused to the C-terminus of said N-intein in said first fusion protein, and said second test polypeptide is fused to the C-terminus of said second inactive reporter polypeptide in said second fusion protein.

20       8.        The method of Claim 2, wherein said first test polypeptide is fused to the N-terminus of said first inactive reporter polypeptide in said first fusion protein, and said second test polypeptide is fused to the C-terminus of said second inactive reporter polypeptide in said second fusion protein.

25       9.        The method of Claim 2, wherein said active reporter protein is detectable by a color assay.

30       10.       The method of Claim 9, wherein said active reporter protein is selected from the group consisting of  $\beta$ -galactosidase, luciferase, green fluorescence protein, blue fluorescence protein, alkaline phosphatase, horseradish peroxidase, and derivatives thereof.

11. The method of Claim 2, wherein said active reporter protein is an auxotrophic protein and is detectable by a cell viability assay.

5 12. The method of Claim 2, wherein the expression of at least one of said fusion proteins in said yeast cell is inducible and occurs only when said yeast cell is subject to a predetermined condition.

10 13. The method of Claim 2, wherein said active reporter protein is a transcription activator and said yeast cell further comprises a detectable gene that is activated when said transcription activator is present.

15 14. The method of Claim 2, wherein said active reporter protein is a transcription repressor and said yeast cell further comprises a detectable gene that is repressed when said transcription repressor is present.

15 15. The method of Claim 2, further comprising introducing into said yeast cell a nucleic acid encoding a third test polypeptide.

20 16. The method of Claim 15, wherein the interaction between said first and second test polypeptide requires the presence of said third test polypeptide.

17. The method of Claim 15, wherein said third test polypeptide modifies post-translationally at least one of said first and second test polypeptides.

25 18. The method of Claim 2, further comprising introducing into the yeast cell a small organic compound to allow said small organic compound to interact with either said first or second test polypeptide or both.

30 19. A method for detecting an interaction between a first test polypeptide and a second test polypeptide, comprising:

conducting a detection assay comprising the steps of (a) producing in a yeast cell a first fusion protein and a second fusion protein, said first fusion protein having said first test polypeptide and an N-intein, said second fusion protein having said second test polypeptide and a C-intein, wherein at least one of the two fusion proteins has an inactive reporter capable of being converted to an active reporter protein upon trans-splicing through said N-intein and said C-intein; and (b) determining the production of said active reporter protein in said yeast cell;

conducting a control assay in which the interaction between the first and second test polypeptides in said fusion proteins in said detection assay is pre-empted; and comparing the level of said active reporter in said detection assay and said control assay.

20. The method of Claim 19, wherein said control assay comprises:  
allowing said first test polypeptide in said first fusion protein to interact with said second test polypeptide in said second fusion protein in the presence of an inhibitor of said interaction; and  
detecting the active reporter.

21. The method of Claim 19, wherein said control assay comprises the steps of:  
producing in a second yeast cell a third and fourth fusion proteins, wherein said third fusion protein is same as said first fusion protein except that said third fusion protein has a third test polypeptide but not said first test polypeptide, said fourth fusion protein is same as said second fusion protein except that said fourth fusion protein has a fourth test polypeptide but not said second test polypeptide, and wherein said third and fourth test polypeptides do not interact with each other; and  
detecting said active reporter protein.

22. The method of Claim 19, wherein said control assay comprises the steps of:

producing in another yeast cell a third fusion protein and a fourth fusion protein, wherein said third fusion protein is same as said first fusion protein except that said third fusion protein lacks said first test polypeptide, said fourth fusion protein is same as said second fusion protein except that said fourth fusion protein lacks said second test polypeptide; and  
5 detecting said active reporter protein.

23. A kit comprising:

a first vector containing a first chimeric gene encoding a first inactive reporter polypeptide fused to the N-terminus of an N-intein and containing an operably linked  
10 first multiple cloning site (MCS) such that when a nucleic acid encoding a first test polypeptide is inserted into said first multiple cloning site, said first chimeric gene is capable of expressing a fusion protein containing said N-intein, said first test polypeptide, and said first inactive reporter polypeptide fused to the N-terminus of said N-intein;  
15 a second vector containing a second chimeric gene encoding a second inactive reporter polypeptide fused to the C-terminus of a C-intein and containing an operably linked second multiple cloning site (MCS) such that when a nucleic acid encoding a second test polypeptide is inserted into said second multiple cloning site, said second chimeric gene is capable of expressing a fusion protein containing said C-intein, said  
20 second test polypeptide, and said second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein; and  
a yeast cell deficient in said active reporter protein.

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24. The kit of Claim 23, wherein said active reporter protein is a functional orotidine-5'-phosphate decarboxylase, said first inactive reporter polypeptide is an N-terminal portion of orotidine-5'-phosphate decarboxylase, said second inactive reporter polypeptide is a C-terminal portion of orotidine-5'-phosphate decarboxylase, and said  
30 yeast cell lacks a functional *URA3* gene.

25. The kit of Claim 23, wherein said active reporter protein is a transcriptional activator, and said kit further comprises a reporting vector having a detectable gene the expression of which is enhanced by said transcriptional activator.

5 26. The kit of Claim 23, wherein said active reporter protein is a transcriptional repressor, and said kit further comprises a reporting vector having a detectable gene the expression of which is repressed by said transcriptional repressor.

27. A kit comprising:  
10 a first expression vector containing a first chimeric gene having from 5' to 3' operably linked in the same open reading frame: (a) a sequence encoding a first inactive reporter polypeptide; (b) a coding sequence for an N-intein; and (c) a first multiple cloning site; and  
a second expression vector containing a second chimeric gene having from 5' to  
15 3' operably linked in the same open reading frame: (a) a second multiple cloning site; (b) a coding sequencing for a C-intein; (c) a sequence encoding a second inactive reporter polypeptide, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter.

20 28. A kit comprising:  
an expression vector containing a chimeric gene having operably linked in the same open reading frame: (a) a sequence encoding a first inactive reporter polypeptide; (b) a coding sequence for an N-intein or C-intein; and (c) a multiple cloning site; and  
25 an expression library expressing a plurality of fusion proteins, each of said fusion proteins having: (a) a polypeptide; (b) a C-intein or N-intein; and (c) a second inactive reporter polypeptide, wherein ligation between said first and second inactive reporter polypeptides forms an active reporter protein.